Exploring the biological mechanism of Wendan Decoction against COPD based on UHPLC-DAD, experimental validation and molecular docking

Qi Yu [a], Guoan Zhao [b], Xuanyu Fu [a], Yuyang Chen [a], Shutong Chen [b], Ying Lyu [c,d], Shan Ren [c,d], Song Lin [c,d,*] Yan Lin [c,d,*], Tianyang Wang [b,*]

Affiliation

[a] Basic Medical Science College, Qiqihar Medical University, Qiqihar, Heilongjiang Province, 161006, China

[b] School of Pharmacy, Qiqihar Medical University, Qiqihar, Heilongjiang Province, 161006, China

[c] Center of Scientific Research, Basic Medical Science College, Qiqihar Medical University, Qiqihar, Heilongjiang Province, 161006, China

[d] Key Laboratory of Homology of Medicine and Food Resources and Metabolic Disease Prevention and Treatment of Heilongjiang Province, Qiqihar, Heilongjiang Province, 161006, China

*These authors were co-corresponding authors.

Correspondence address

Song Lin, E-mail: linsong0228@163.com; Yan Lin, E-mail: linyan_qqhr@163.com; Tianyang Wang, E-mail: tanya.sunny@163.com

Phone: (+86) 0452 2663067. Fax: (+86) 0452 2663067.

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List of Supplementary Materials

1 Gradient elution conditions

Table S1 The program of gradient elution

t /min	Φ (mobile phase A) /%	Φ (mobile phase B) /%
0-1	90-92	8-10
1-6	92-86	8-14
6-8	86-84	14-16
8-10	84-80	16-20
10-15	80-80	20-20
15-22	80-75	20-25
22-25	75-70	25-30
25-30	70-60	30-40
30-35	60-40	40-60
35-37	40-10	60-90
37-42	10-10	90-90
42-42.01	10-2	90-98

2 Solution preparation

Preparation of Test Solution detailed procedures: According to the traditional decocting process of TCM, accurately weigh 6 g of Banxia (dried tuber of *Pinellia ternata* (Thunb.) Breit.), 9 g of Jupi (dried and ripe peel of *Citrus reticulata Blanco*), 6 g of Zhuru (the part under the skin of *Bambusa tuldoides* Munro), 6 g of Zhishi (dried unripe fruit of *Citrus aurantium* L.), 12 g of Shengjiang (fresh rhizome of *Zingiber officinale* Rosc.) and 3 g of Gancao (dried roots and rhizomes of *Glycyrrhiza uralensis* Fisch.). Add pure water at a ratio of 1:8 (w/v, crude drug mass: water volume, g: mL), soak at room temperature for 30 min, then decoct twice (20 min each time). Filter with sterile gauze, combine the two extracts to prepare the crude WDD extract. Before the experiment, centrifuge the crude extract at a speed of 10,000 rpm for 5 min. Take the supernatant and filter it through a 0.22 μm pore size membrane. Discard the initial filtrate and take the subsequent filtrate as the Test Solution for UHPLC analysis. Prepare negative control solutions without Gancao, Jupi, Shengjiang, and Zhishi, respectively, in the same way, which are used for the subsequent validation of component specificity.

Preparation of Reference Substance Solution: Precisely weigh 11 reference substances, respectively, place them in a 10 mL volumetric flask, and dissolve them with methanol to prepare a mixed reference substance stock solution containing 28.4 μg of liquiritin, 125.4 μg of naringin, 386.2 μg of hesperidin, 885.0 μg of neohesperidin, 5.1 μg of isoliquiritin, 27.0 μg of naringenin, 6.2 μg of apigenin, 105.9 μg of 6-gingerol, 20.8 μg of 8-gingerol, 15.6 μg of 10-gingerol, and 10.2 μg of glycyrrhetinic acid per 1 mL.

3 Specific primer sequences used in qRT-PCR

Table S2 Specific primer sequences used in qRT-PCR

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
IL-1β	CAGCTTTCGACAGTGAGGAGA	TGTCGAGATGCTGCTGTGAG
IL-18	AGAACCAATGCGAGATCCTGACAAC	GTCACAGCCAGTCCTCTTACTTCAC
NLRP3	GAGCTGGACCTCAGACAATGC	AGAACCAATGCGAGATCCTGACAAC
Caspase-1	GACCGAGTGGTTCCCTCAAG	GACGTGTACGAGTGGGTGTT
COX-2	CTGTTCCAACCCATGTCAAAACC	GTACAGTTTTCACCGTAGAATCCA
PLA2	CCGGGAGTGATCCCCTGAA	ACCTGTCTAAGTCGTCCACTG
NF-κB	TCTACAGGCAGAAGGCGGAGGA	TGGCGTCTGACACCACAGGTTC
GAPDH	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT

4 Key identification data of ligands and receptors in molecular docking

Table S3 UniProt IDs of target proteins used in molecular docking

Target protein	AlphaFold ID	UniProt ID
NF-κB	AF-Q63369-F1-v6	Q63369
NLRP3	AF-D4A523-F1-v6	D4A523
PLA2	AF-P51433-F1-v6	P51433
COX-2	AF-P00406-F1-v6	P00406

Table S4 PubChem IDs of 11 representative components used in molecular docking

Analytes	PubChem ID
Liquiritin	503737
Naringin	442428
Hesperidin	10621
Neohesperidin	30231
Isoliquiritin	5318591
Naringenin	439246
Apigenin	5280443
6-Gingerol	34756
8-Gingerol	168114
10-Gingerol	168115
Glycyrrhetinic acid	636403

5 Similarity analysis results of UHPLC-DAD fingerprint

Table S5 RSD of relative retention time and relative peak area of characteristic peaks

Analytes	RSD%(RRT)	RSD%(RPA)
Liquiritin	0.24	29.51
Naringin	0.73	5.32
Hesperidin	0.78	14.68
Neohesperidin	0.00	0.00
Isoliquiritin	2.97	17.91
Naringenin	0.00	0.00
Apigenin	0.15	41.25
6-Gingerol	0.09	11.37
8-Gingerol	1.03	21.70
10-Gingerol	1.13	32.17
Glycyrrhetinic acid	0.95	12.02

6 Assignment of characteristic peaks of WDD

Table S6 Results of medicinal ingredient peak assignment for WDD

Peak number	Component	Medicinal ingredient assignment
1	Liquiritin	Gancao
2	Neohesperidin	Zhishi
4	Naringenin	Zhishi
5	Isoliquiritin	Gancao
6	Naringin	Zhishi
7	Apigenin	Jupi
8	6-Gingerol	Shengjiang
9	8-Gingerol	Shengjiang
10	10-Gingerol	Shengjiang

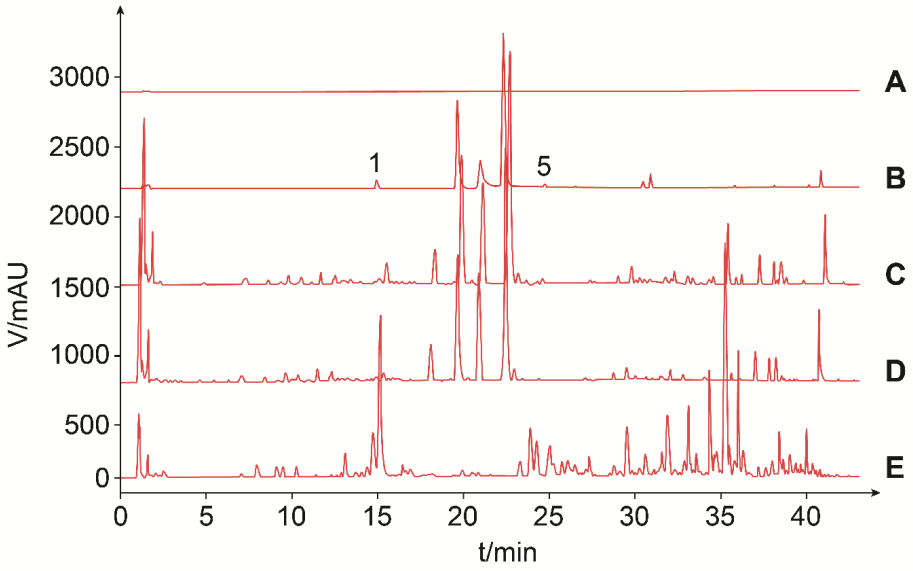

Fig. S1 (A) Methanol negative control solution in 254 nm. (B) Mixed reference standard solution in 254 nm. (C) Test solution in 254 nm. (D) Deficiency of Gancao test solution in 254 nm. (E) Gancao single herb medicine in 254 nm.

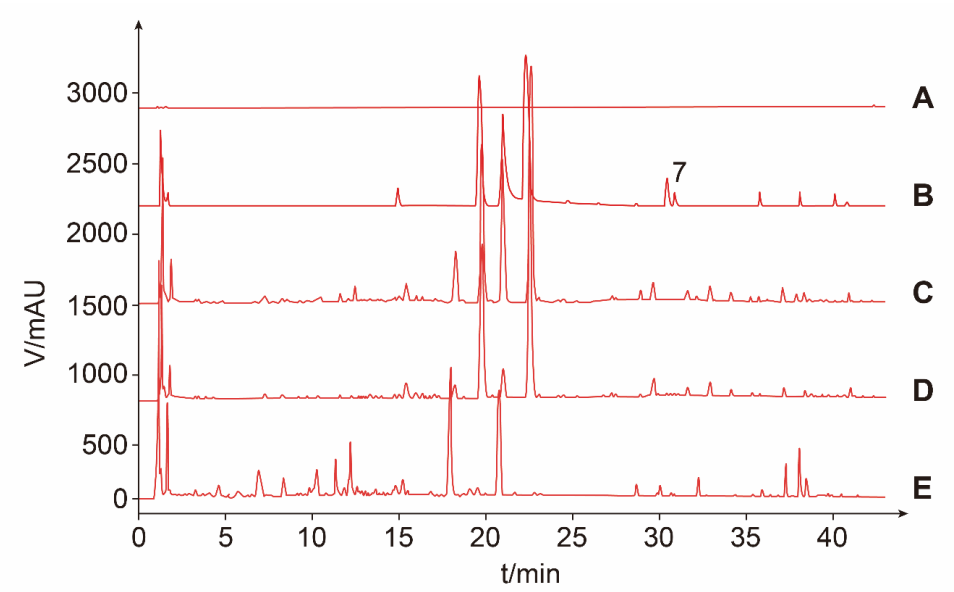

Fig. S2 (A) Methanol negative control solution in 215 nm. (B) Mixed reference standard solution in 215 nm. (C) Test solution in 215 nm. (D) Deficiency of Jupi test solution in 215 nm. (E) Jupi single herb medicine in 215 nm.

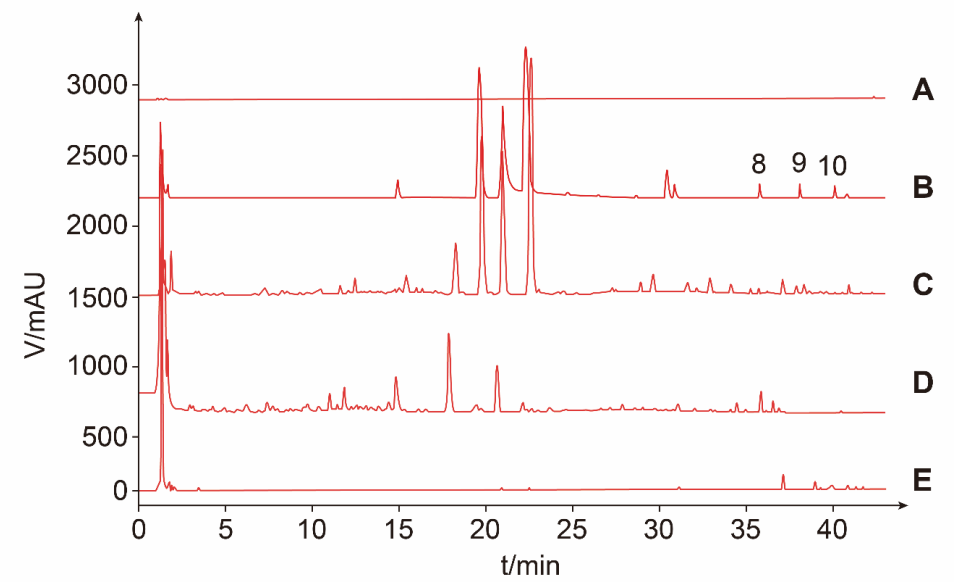

Fig. S3 (A) Methanol negative control solution in 215 nm. (B) Mixed reference standard solution in 215 nm. (C) Test solution in 215 nm. (D) Deficiency of Shengjiang test solution in 215 nm. (E) Shengjiang single herb medicine in 215 nm.

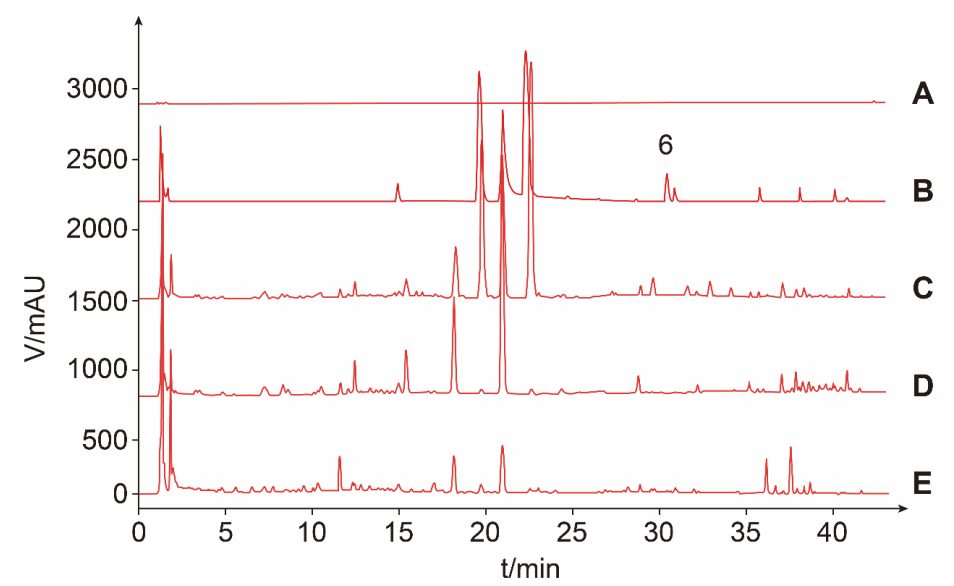

Fig. S4 (A) Methanol negative control solution in 215 nm. (B) Mixed reference standard solution in 215 nm. (C) Test solution in 215 nm. (D) Deficiency of Zhishi test solution in 215 nm. (E) Zhishi single herb medicine in 215 nm.

7 Method validation results for 11 analytes in WDD

Table S7 Accuracy validation results of chromatographic conditions for 11 analytes

Analytes	Original (μg)	Spiked (μg)	Found (μg)	Recovery (%)	RSD (%)
Liquiritin	284.22	142.11	289.35	104	0.58
		284.22	278.47	98	0.92
		426.33	249.19	92	1.83
Naringin	1254	627	1218.01	94	1.14
		1254	1282.84	102	1.22
		1881	1303.69	103	0.23
Hesperidin	3862.647	1931.3235	3897.23	102	0.59
		3862.647	3874.31	100	0.27
		5793.9705	3459.43	93	9.54
Neohesperidin	8850.196	4425.098	8992.08	103	1.27
		8850.196	9002.91	102	0.14
		13275.294	8410.80	97	1.21
Isoliquiritin	51.248	25.624	50.45	97	0.97
		51.248	53.84	105	3.30
		76.872	49.99	98	0.75
Naringenin	270.069	135.03	267.46	98	0.34
		270.07	270.07	100	0.77
		405.10	262.41	98	1.23
Apigenin	62.232	31.12	62.55	101	0.60
		62.23	62.07	100	0.92
		93.35	63.35	101	0.97
6-Gingerol	1058.548	529.27	1019.90	93	1.62
		1058.55	1000.89	95	1.94
		1587.82	951.19	93	0.30
8-Gingerol	207.729	103.86	203.31	96	0.80
		207.72	208.89	101	0.38
		311.59	202.93	98	0.37
10-Gingerol	155.657	77.83	157.07	102	0.26
		155.66	157.47	101	1.02
		233.49	151.68	98	0.69
Glycyrrhetinic acid	101.54	50.77	97.17	91	0.22
		101.54	101.97	100	1.38
		152.31	91.52	93	3.52

Table S8 Repeatability results of chromatographic conditions for 11 analytes

Analytes	RSD% (50% Content)	RSD% (100% Content)	RSD% (150% Content)
Liquiritin	0.49	0.93	0.58
Naringin	0.15	0.88	0.19
Hesperidin	0.83	0.48	0.82
Neohesperidin	0.79	0.10	0.88
Isoliquiritin	0.72	0.95	0.40
Naringenin	0.44	0.29	0.35
Apigenin	0.20	0.36	0.41
6-Gingerol	0.63	0.19	0.89
8-Gingerol	0.32	0.81	0.85
10-Gingerol	0.37	0.46	0.80
Glycyrrhetinic acid	0.55	0.21	0.14

Table S9 Intermediate precision results of chromatographic conditions for 11 analytes

Analytes	Distinct analysts (%)	Distinct analysis dates (%)	Distinct instruments (%)
Liquiritin	0.36	0.76	0.91
Naringin	0.94	1.61	1.48
Hesperidin	0.86	0.44	0.94
Neohesperidin	1.24	1.07	1.19
Isoliquiritin	1.47	0.71	0.92
Naringenin	0.34	0.92	0.25
Apigenin	1.11	0.85	0.49
6-Gingerol	0.22	0.89	0.96
8-Gingerol	0.78	1.20	0.53
10-Gingerol	0.99	0.85	0.95
Glycyrrhetinic acid	1.27	1.06	1.26

Table S10 Chromatographic condition durability test results of 11 analytes at 254 nm

No.	Factors	Content /%				
		Liquiritin	Naringin	Hesperidin	Neohesperidin	Isoliquiritin
1	Injection volume (IV)	0.42	0.84	0.16	0.90	0.76
2	Wave length (WL)	0.47	0.39	0.55	0.27	0.98
3	Flow rate (FR)	1.05	0.17	0.19	0.92	1.23
4	Acid concentration-H_3PO_4	0.29	0.59	1.21	0.62	1.39
5	Col. temp (CT)	0.35	0.64	0.88	0.19	0.85
6	Ratio of organic phase (WP)	0.47	1.05	0.85	0.64	0.46
7	Time of gradient (TP)	0.46	0.53	0.37	0.20	1.52

Table S11 Chromatographic condition durability test results of 11 analytes at 215 nm

No.	Factors	Content /%					
		Naringenin	Apigenin	6-Gingerol	8-Gingerol	10-Gingerol	Glycyrrhetinic acid
1	Injection volume (IV)	0.45	0.38	0.26	0.54	0.46	1.47
2	Wave length (WL)	0.14	0.56	0.08	0.06	0.50	1.34
3	Flow rate (FR)	0.31	0.38	1.45	0.41	0.61	0.57
4	Acid concentration-H_3PO_4	0.36	0.78	0.13	0.36	1.21	0.42
5	Col. temp (CT)	0.12	0.93	0.38	0.08	0.81	0.19
6	Ratio of organic phase (WP)	1.73	0.92	0.87	0.41	0.31	1.39
7	Time of gradient (TP)	0.34	0.50	1.80	0.90	1.35	1.36

Table S12 Solution stability results of 11 analytes under chromatographic conditions

Peak number	Analytes	Analytes
1	Liquiritin	0.35
2	Naringin	0.75
3	Hesperidin	0.28
4	Neohesperidin	0.61
5	Isoliquiritin	1.02
6	Naringenin	0.90
7	Apigenin	0.43
8	6-Gingerol	0.87
9	8-Gingerol	0.87
10	10-Gingerol	0.78
11	Glycyrrhetinic acid	0.71

Table S13 The content determination results of 11 analytes

Peak number	Analytes	Content (mg/g)
1	Liquiritin	0.284 ± 0.009
2	Naringin	1.254 ± 0.016
3	Hesperidin	3.862 ± 0.052
4	Neohesperidin	8.850 ± 0.020
5	Isoliquiritin	0.051 ± 0.001
6	Naringenin	0.270 ± 0.008
7	Apigenin	0.062 ± 0.008
8	6-Gingerol	1.058 ± 0.005
9	8-Gingerol	0.207 ± 0.004
10	10-Gingerol	0.155 ± 0.013
11	Glycyrrhetinic acid	0.101 ± 0.019

8 Therapeutic efficacy of WDD on COPD rats

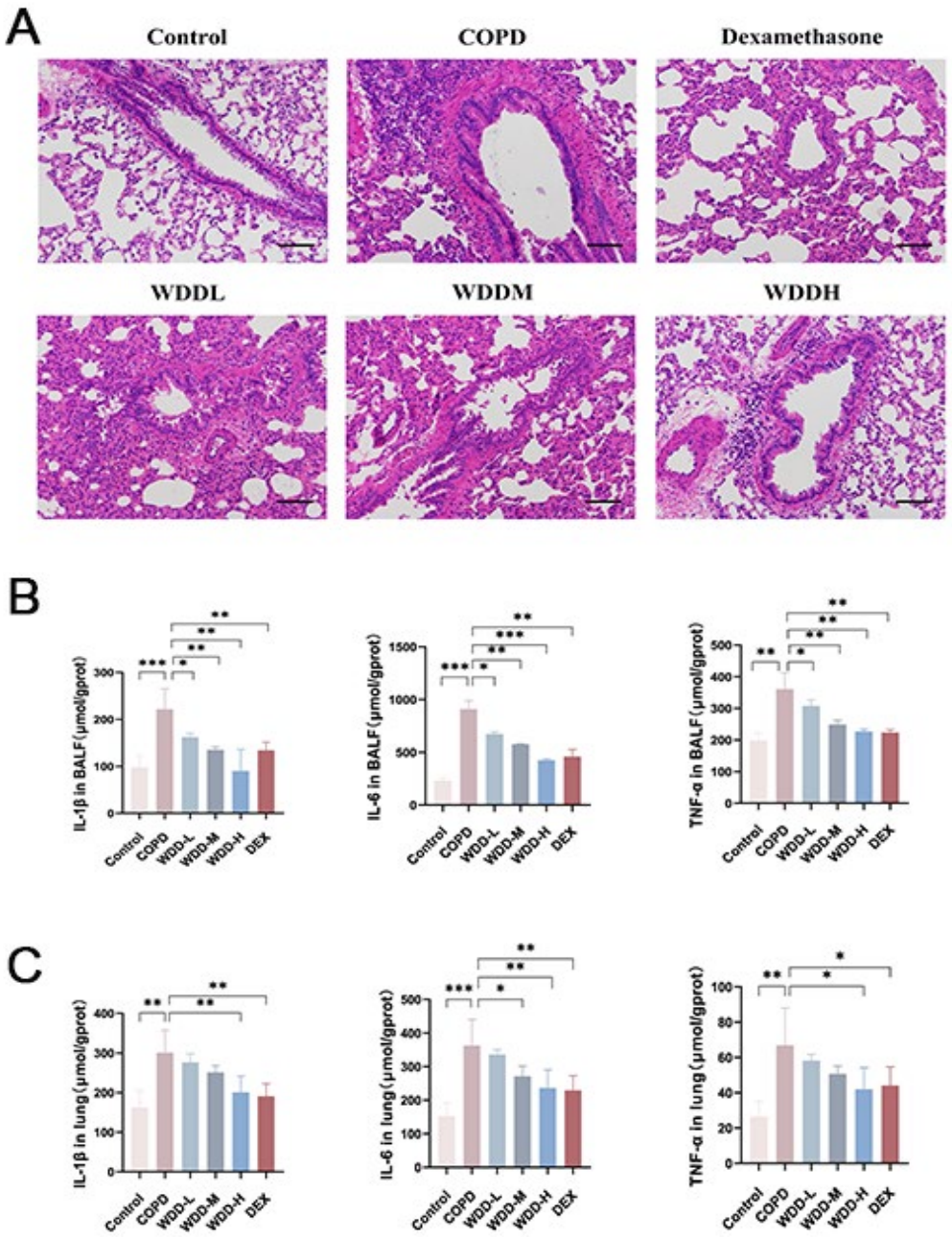

Fig. S5 (A) Therapeutic efficacy of WDD on COPD rats H&E staining (×200 magnification). (B, C) ELISA results of IL-1β, IL-6 and TNF-α in the BALF and lung (mean ± SD, n = 3, ∗ $p < 0.05$ vs COPD group; ∗∗ $p < 0.01$ vs COPD group; ∗∗∗ $p < 0.001$ vs COPD group).

9 LC-MS detection results of AA and LTE_3

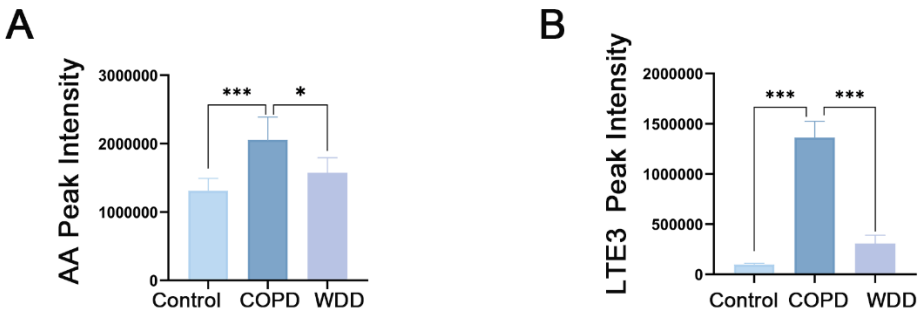

Fig. S6 (A) AA peak intensity; (B) LTE_3 peak intensity. LC-MS-based quantification of AA and LTE_3 in lung tissue/BALF samples, expressed as peak intensity.

10 Molecular docking analysis

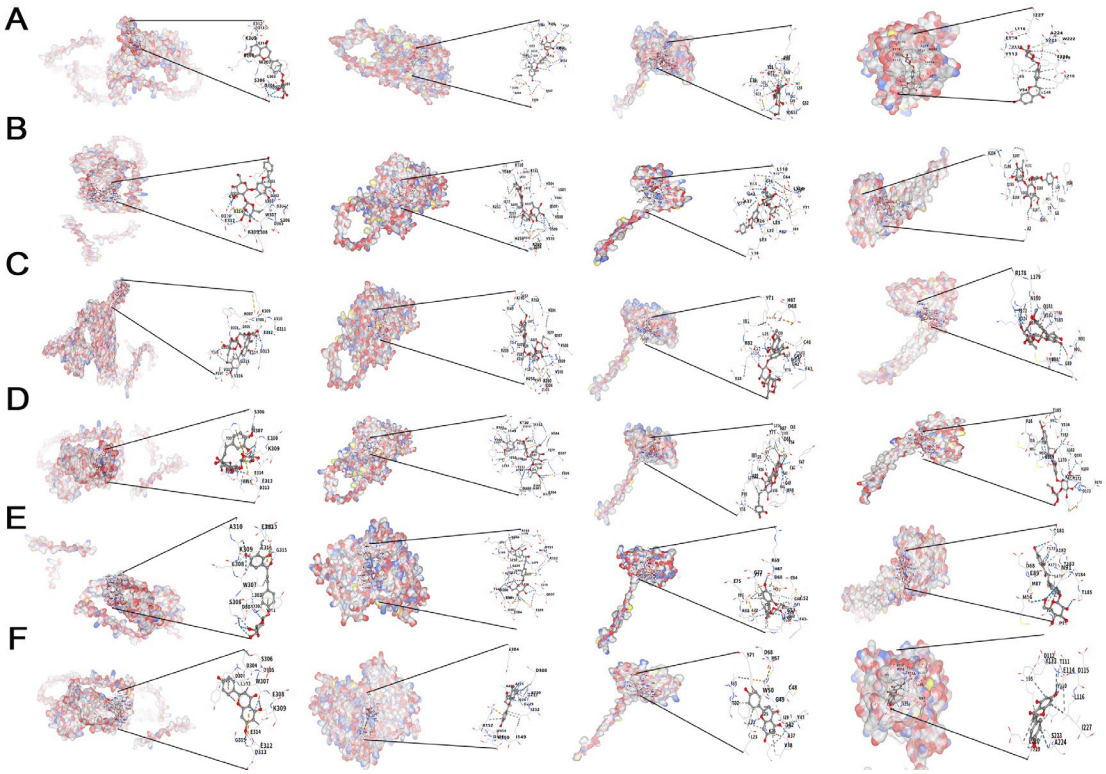

Fig. S7 (A) CB-Dock2 display the docking results of Liquiritin with NF-κB, NLRP3, PLA2 and COX-2. (B) CB-Dock2 display the docking results of Naringin with NF-κB, NLRP3, PLA2 and COX-2. (C) CB-Dock2 display the docking results of Hesperidin with NF-κB, NLRP3, PLA2 and COX-2. (D) CB-Dock2 display the docking results of Neohesperidin with NF-κB, NLRP3, PLA2 and COX-2. (E) CB-Dock2 display the docking results of Isoliquiritin with NF-κB, NLRP3, PLA2 and COX-2. (F) CB-Dock2 display the docking results of Naringenin with NF-κB, NLRP3, PLA2 and COX-2.

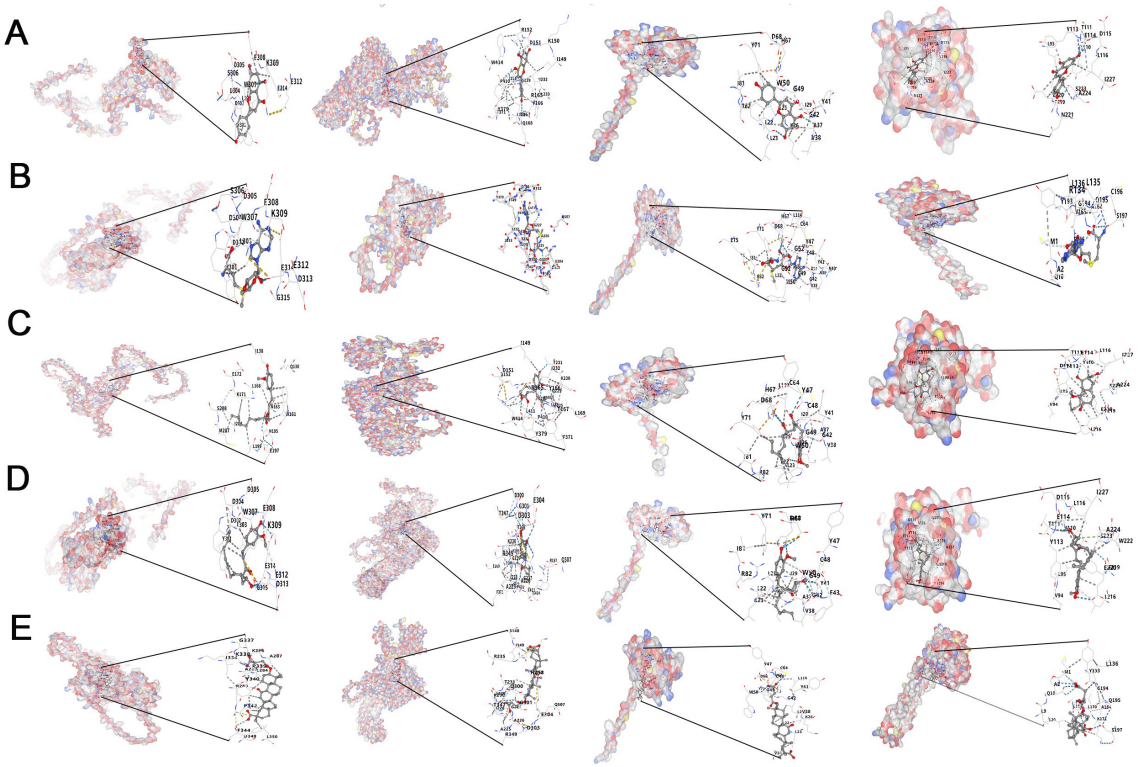

Fig. S8 (A) CB-Dock2 display the docking results of Apigenin with NF-κB, NLRP3, PLA2 and COX-2. (B) CB-Dock2 display the docking results of 6-Gingerol with NF-κB, NLRP3, PLA2 and COX-2. (C) CB-Dock2 display the docking results of 8-Gingerol with NF-κB, NLRP3, PLA2 and COX-2. (D) CB-Dock2 display the docking results of 10-Gingerol with NF-κB, NLRP3, PLA2 and COX-2. (E) CB-Dock2 display the docking results of Glycyrrhetinic acid with NF-κB, NLRP3, PLA2 and COX-2.